Research Article

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Efficacy of Novel Antimicrobial Therapies for Treating *Staphylococcus* Infections in Wistar Rats

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Abstract

The novel semisynthetic lipoglycopeptide dalbavancin has enhanced antibacterial action against a wide range of gram-positive bacteria. This current study was conducted to evaluate the efficacy of dalbavancin in treating pathogenic Staphylococcus infections in Wistar rats. The experiment included 20 animals, divided into 2 groups, a control group and an experimental group. All rats were anaesthetized and had a Kirschner wire plus clinical methicillin-resistant Staphylococcus aureus (MRSA) isolate placed into their proximal tibia. The experimental group (10 rats) were treated with a loading dosage of dalbavancin (20 mg/kg) administered throughout 4 weeks following the induction, while the control group (10 rats) were left untreated. Microbiological analysis was performed on tibial bones and Kirschner wires within 24 hours after the last dosage was given. In comparison with the control group, the dalbavancin-treated group showed improvement in quantitative bacterial cultures of osseous tissue. After four weeks of treatment with dalbavancin, there were no signs of induced glycopeptide-/lipoglycopeptide-resistant strains. In conclusion, this study confirmed that treatment with dalbavancin was effective in the treatment of experimental implant-related MRSA osteomyelitis. However, the therapeutic effectiveness of dalbavancin in treating S. aureus infections still needs further investigation; ideally, it should be studied in conjunction with biofilm-active medicines.

Keywards: Dalbavancin, Osteomyelitis, Methicillin-Resistant *Staphylococcus aureus* (MRSA), Wistar Rats.

فعالية العلاجات المضادة للميكروبات الجديدة في علاج عدوى المكورات العنقودية في الجرذان نوع ويستار

الخلاصة

أثبت عقار الدالبافانسين (dalbavancin) الدهني الشحمي شبه الاصطناعي الجديد ان له تأثير معزز مضاد للبكتيريا ضد مجموعة واسعة من انواع البكتيريا إيجابية صبغة جرام. أجريت هذه الدراسة لتقييم فعالية الدالبافانسين في علاج الاصابة بعدوى المكورات العنقودية الذهبية المقاومة للميثيسيلين methicillin-resistant *Staphylococcus aureus* (MRSA) في الجرذان من فصيلة ويستار والمحقونة مسبقا بالعزلات البكتيرية المرضية . شملت التجربة عشرون حيواناً، من بعد تخدير كل جرذ، وباستخدام سلك كيرشنر وعزلات سريرية من MRSA حقنت في الجزء الداني لعظم الساق (الظنبوب القريب) proximal tibia. نصف الحيونات (10 جرذان) تم إعطاؤهم الدالبافانسين بجرعة تحميل 20 ملغم/كغم/يوم على مدار أربعة أسابيع بعد الإصابة كمجموعة اختبارية، أما النصف الاخر (10 جرذان) تركت دون معالجة كمجموعة سيطرة. بعد انتهاء فترة التجربة تم إجراء تحليل ميكروبيولوجي على عظام الظنبوب وأسلاك كيرشنر، بعد 24 ساعة من إعطاء الجرعة الأخيرة في اليوم الاخير من الاسبوع الرابع. تبين من خلال النتائج ان مجموعة التجربة قد أظهرت بأن المعالجة بالدالبافانسين منحتهم تحسناً معنويا في المزارع البكتيرية الكمية للأنسجة العظمية، ولم تظهر أي علامة على وجود سلالة مقاومة للجليكوبتيد/الليبوجليكوببتيد، بالمقارنةً مع العزلات المأخوذة من مجموعة السيطرة. في الختام، أكدت هذه الدراسة أن العلاج بالدالبافانسين كان فعالاً في علاج التهاب العظم والنقي التجريبي المرتبط بالاصابة بالعزلات المرضية من مكورات MRSA. ومع ذلك، فإن الفعالية العلاجية للدالبافانسين كعلاج مضاد لعدوى المكورات العنقودية الذهبية مازال يحتاج إلى مزيد من الدراسات والتحقيق في المستقبل؛ فمن المثالي مقارنة استخدام علاج الدالبافانسين بالتزامن مع الأدوية النشطة ضد الأغشية الحيوية المختلفة.

Introduction:

The Gram-positive cocci that produce catalase and have sizes between 0.5 and 1.5 μm are members of the Staphylococcus spp., which derived from the Greek words staphyle meaning "a bunch of grapes" and kokkos meaning "grain or berry." This family is known as Staphylococcaceae. Sir Alexander Ogston first linked the bacteria Staphylococcus to a suppurative abscess in a knee joint in the 1880s, when the word was originally coined (1). Bacteria may be found individually, in pairs, tetrads, or short chains that form clusters that resemble grapes; they are neither motile nor spore-forming (2). Staphylococcus species have traditionally been categorised as either coagulase-positive or coagulase-negative based on their capacity to manufacture coagulases in a controlled laboratory setting (1).

When it comes to infections in humans, the *Staphylococcus aureus* (*S. aureus*) is by far the most common coagulase-positive staphylococcus (3,4). The anterior nostrils are the most common place for this bacterium to colonise, however it is a common member of the human microbiota and may be found anywhere in the body (5). The temperature range for this type of facultative non-fastidious anaerobe bacteria is 15–45 °C, and it can thrive in NaCl concentrations as high as 15%. Staphyloxanthin is a yellow pigment that gives *S. aureus* its characteristic golden colour on rich medium like tryptic soy agar. However, when grown on blood agar, whether from sheep or rabbits, it produces a hemolytic halo. Being able to thrive in environments with high salt concentrations and ferment mannitol to produce acid has led to its usage as a selective and differential medium in mannitol salt agar with 7.5% NaCl (6). *S. aureus* has the remarkable capacity to develop resistance to almost all antimicrobials. Thus, it was not long after the beta-lactam antibiotics penicillin (7) and methicillin (8) were first used in clinical practice that resistance to these drugs began to develop. A combination of mutations impacting essential metabolic processes and the acquisition of mobile genetic elements (MGE) including resistance-encoding genes has been associated with S. aureus's antibiotic resistance. These occurrences may cause a variety of resistance mechanisms, such as the inactivation of drugs by enzymes, changes to the target, active protection of the target site, and antimicrobial efflux (9,10,11).

The lipoglycopeptide antibiotic dalbavancin was structurally developed from a naturally occurring glycopeptide by *Nonomuria spp*. that resembles teicoplanin (12). Acute Gram-positive bacterial infections of the skin and soft tissues in humans may be treated with this drug. Dalbavancin and vancomycin both work by binding to and inhibiting D-alanyl-D-alanine, but dalbavancin contains an additional lipophilic side chain that attaches to bacterial cell membrane lipid II. Its lengthy half-life and strong plasma protein binding make weekly dosage possible (13).

According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the staphylococci susceptibility breakpoint is ≤0.125 mg/L (14). Researchers in 2009 compared the in vitro activity of dalbavancin to that of vancomycin, finding that it had MIC90 values of 0.06 mg/L against 27,052 MSSA bacteria and 19,721 MRSA strains, compared to 1 mg/L for vancomycin (15).

The identical dalbavancin MIC50 and MIC90 values of 0.06 mg/L were obtained in several later experiments that included both MSSA and MRSA strains (16-17). One possible mechanism by which dalbavancin aids in *S. aureus* infection management is by inhibiting toxin synthesis in vitro (18). Last but not least, dalbavancin exhibits great penetration into

synovial fluid and tissue, bone, skin, and blister fluid (19) and blister fluid (20), suggesting significant local concentrations that may aid in infection management and eradication.

Therefore, this study has conducted to evaluate the efficacy of dalbavancin in treating pathogenic Staphylococcus infections in Wistar rats.

Materials and Methods:

Animals

In a climate- and light-controlled environment, twenty adults male Wistar rats weighing 180–230 g were kept. Everything from food to drink was freely provided. These animals divided randomly into control group (G1) and experimental group (G2). Each group consists of 10 animals.

Bacterial strain:

A clinical pathogenic strain of MRSA used, which was previously isolated from cases of osteomyelitis. The bacterial isolates were cultured in Tryptic Soy Broth (TSB) overnight, then diluted 1:100 in new TSB. To get them to the exponential growth phase, they were incubated at 37°C for five hours.

The broth microdilution technique used to count the MIC of dalbavancin in Mueller-Hinton broth (CAMHB) with glucose-6-phosphate added, as a final concentration of 25 mg/liter (22). Samples of MRSA isolates were taken from treated bones at the last day of the experiment.

Dalbavancin preparation

The dalbavancin has prepared as directed by the manufacturer by diluting them with 5% dextrose solution.

Dosage recommendations derived from drug concentrations in osseous tissue of rats that were shown to be highly indicative of human bone concentrations in earlier investigations (21).

Surgical procedure:

The left proximal tibia of every animal was perforated with a Kirschner-wire that measured 1 cm in length and an inoculum containing 10 μL of the bacterial suspension (1.5×10^6 CFU/bone). Viable count validation was performed both before and after surgical operations to ensure accurate bacterial counts.

Animals were having radiographic evidence of tibial osteomyelitis. They randomly randomized to receive intraperitoneal dalbavancin 20 mg/kg (G2) or no treatment therapy given (G1) for four weeks after the infection have induced.

Statistical analysis

The SPSS (Statistical Package for the Social Sciences) software used to analyse all collected data.

Results and Discussion:

Cultures of bacteria from Kirschner-wires and bone showed a tested positive for MRSA in all animals. Figure 1 shows that the bacterial counts in osseous tissue for animals treated with dalbavancin were 5.82 $\log_{10}$ CFU/g bone, whereas for animals that were not treated, the bacterial counts were 6.41 $\log_{10}$ CFU/g bone. The dalbavancin-treated group (G2) differed significantly from the control group (G1) that did not receive therapy (Table 1, Figure 1).

Dalbavancin exhibited minimum inhibitory concentrations (MICs) of 0.063 mg/L in all isolates tested, including that are derived from Kirschner-wires as well as antibiotic-treated bone samples. Thus, induced glycopeptide/lipoglycopeptide resistance did not develop in our investigation.

Table 1. Mean bacterial count in log_{10} CFU/g bone in studied groups

Groups	Mean log_{10}	SE
G1	6.41 A	0.14
G2	5.82 B	0.21

Different letters denote differences between studied groups

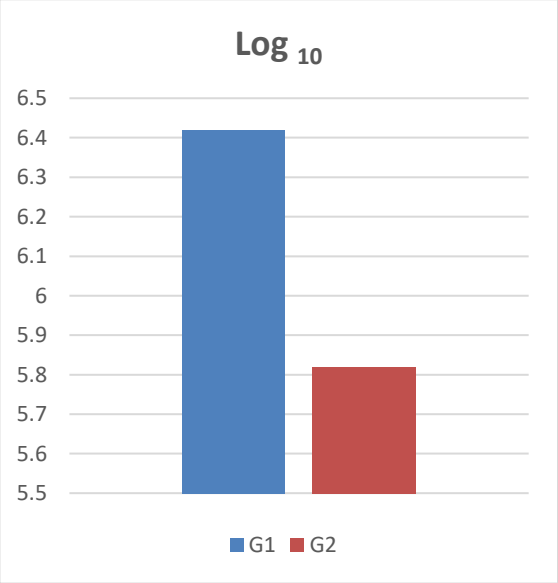

Figure 1 shows the mean of bacteria in log_{10} CFU/g of bone for each group.

The current investigation found that dalbavancin is effective as treatment to the MRSA infected rats. This finding is agreed to Barnea *et al*. who found that a >3 log_{10} CFU/g bone reduction in sternal MRSA osteomyelitis rats treated with dalbavancin, although there was no foreign body inserted to the bone, which usually increase bacterial burdens in the osseous tissues of the osteomyelitis model, thus, antibiotic treatment was more successful (21). Other researchers conduct previous experiments, in both laboratory settings (*in-vitro*) and living organisms (*in-vivo*), have declared that antibiotic doses required to eradicate biofilms are 10–1000 times higher than planktonic cell MICs or MBCs (23,24,25,26). When trying to eradicate biofilms, these concentrations are often too high, especially in poor penetration compartments like osseous tissue (23,26).

The median bacterial counts of implanted wires in the treated groups were 7.50×10^2 CFU/implant (0 to 7×10^3), whereas in the untreated control groups they were only 2.5×10^3 CFU/implant (4.0×10^2 to 4.5×10^4), according to earlier investigations (27, 28). So, the elevated bacterial counts seen in the osseous tissue in this investigation probably weren't caused only by contamination via the implanted wires. Therefore, current study findings of dalbavancin monotherapy's poor effectiveness may be best if a fully developed biofilm forms on the implanted Kirchhoff wires.

People who need to take antibiotics for a long time, often given 500 to 1000 mg of dalbavancin once a week (29). The presence of dalbavancin concentrations in osseous tissue suggests that there are enough drug levels to treat organisms that are sensitive to dalbavancin. The goal of the treatment plans utilised in this research to have the meditational concentrations in the bones that are, as close as possible, similar to what would be advised to people (drug concentration/kg). Following a loading dose of 20 mg/kg and a maintenance dose of 10 mg/kg, rats exhibited bone levels comparable to an intravenous administration of 1000 mg of dalbavancin in humans (21,29).

It should be noted, nonetheless, that the current investigation did not measure dalbavancin concentrations in the bones, which were presumed to have reached levels comparable to those investigated previously (21). Curiously, during a four-week treatment period, no signs of dalbavancin-resistant *S. aureus* isolates were seen. The development of resistance to dalbavancin is very unusual, as previously noted (30). Vancomycin and teicoplanin are currently recommended as first-line treatments

for MRSA infections that might occur after getting an orthopaedic implant (31).

Conclusion:

In a rat study, dalbavancin effectively treated experimental implant-related MRSA osteomyelitis. The therapeutic effectiveness of dalbavancin in treating *S. aureus* infections needs more investigation; ideally, it should be studied in conjunction with biofilm active medications.

Conflict of interest:

Regarding this manuscript's publication and financing, the authors state that they have no conflicts of interest.

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